# Blood Human Chorionic Gonadotropin (hCG) Assays: What Laboratorians Should Know about False-Positive Results

## 1. What Are the Scope and Purpose of This Communication?

The Food and Drug Administration's (FDA) Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD) has developed this communication in response to reports sent to FDA by manufacturers and users of hCG assays of adverse events associated with falsely elevated human chorionic gonadotropin (hCG) results. This phenomenon has also been described in the scientific literature. hCG false-positive results have led to errors in patient diagnosis and treatment with severe clinical consequences including unnecessary interventions such as chemotherapy and major surgical procedures. In this document, an hCG false-positive result is defined as the detection of hCG by immunoassay in the absence of actual hCG in blood.

The purpose of this communication is: **a)** to remind **laboratorians** and clinicians of analytical interfering factors and clinical conditions that may affect the performance of the hCG immunoassay testing, **b)** to provide techniques to identify any suspected interference, and **c)** to recommend methods to reduce or eliminate the interfering factors.

## 2. What Is hCG and What Does hCG Immunoassay Measure?

hCG is a glycoprotein consisting of two dissimilar subunits (a and ß) with eight carbohydrate side chains. This combination results in significant heterogeneity in the hCG structure.[1] The hCG molecule is produced by trophoblast cells of the placenta; by trophoblast cells in gestational trophoblastic diseases such as hydatidiform moles, choriocarcinoma, and placental site trophoblastic tumors; and by trophoblast cells in testicular germ cell malignancies.[1,2]

The gonadotropes[i] in the human pituitary also synthesize and secrete hCG (known as pituitary hCG), but this molecular form is slightly different from placental hCG.[1] Many nontrophoblastic tumors also express hCG.[3]

hCG immunoassays measure the presence (qualitative) or amount (quantitative) of hCG molecule- regular and variant molecular forms- in blood or urine. All hCG immunoassays are based on the sandwich principal; at least one antibody binds and immobilizes hCG and a second antibody raised to a distant epitope and labeled with an enzyme, dye, or chemilluminescence agent, marks the presence of hCG or quantifies it.[2,4] Most hCG assays are designed to primarily detect the regular placental form of the hCG molecule since regular hCG is considered to be the key marker for pregnancy.[2]

## 3. What Clinical Conditions Are Associated with Detectable Levels of hCG?

[i] **Gonadotropes** are cells in the anterior pituitary which produce the gonadotropin luteinizing hormone and follicle stimulating hormone.

In non-pregnant women, hCG levels are normally undetectable. During early pregnancy, the placenta produces hCG and its level in the blood doubles every two to four days.[5] Low levels of hCG are present following an early, spontaneous abortion. In gestational trophoblastic diseases, serum hCG is persistently elevated. However, one to five years *preceding* malignant gestational trophoblastic disease, persistently low levels of hCG (e.g., <50 IU/L) may be present.[6]

Pituitary hCG circulates in low concentrations in men and premenopausal women, and the concentrations rise in perimenopausal women and older women.[1] A variable proportion of many nontrophoblastic tumors, such as transitional cell carcinoma of the bladder and urinary tract, renal cancer, prostate cancer, cancers of the gastrointestinal system, neuroendocrine tumors, lung cancer, breast cancer, gynecological cancers, and hematological cancers, also express hCG at various levels.[3]

## 4. What Is the Intended Use of hCG Immunoassays?

hCG immunoassays are intended to measure hCG, a placental hormone, in blood or urine for the early detection of pregnancy. Other uses include aiding in the diagnosis, prognosis, management, and treatment of certain tumors. The level of regulatory oversight of these assays varies with the intended use of the device. When they are used for the early detection of pregnancy, they are regulated as Class II devices. When they are intended for uses other than early detection of pregnancy, they are regulated as Class III devices.[7]

FDA has cleared a large number of hCG assays (professional and home use) for the early detection of pregnancy. The diagnosis and monitoring of early pregnancy is the only application for hCG assays that is cleared by FDA although the performance of these assays has not been established in association with ectopic pregnancy or molar pregnancy. Even though not approved by FDA, the measurement of hCG for the diagnosis and monitoring of ectopic pregnancy, molar pregnancy, gestational trophoblastic diseases[1,3,4,8], and testicular cancer[9], is considered the standard of care in the United States.

## 5. What Factors Can Cause False-Positive hCG Assay Results?

Clinically significant hCG false-positive results are rare but do occur.[10] False-positive results may occur because of analytical interfering factors and/or device malfunctions. Therefore, it is important that laboratorians, working in conjunction with physicians who order hCG tests, are aware of the underlying causes of falsely elevated results so that they can assist physicians to properly utilize hCG results in the patient management.

Since device malfunctions have broad potential sources and root causes, it is outside the scope of this document to address them. However, the analytical interfering factors are extensively discussed below.

## 6. What Are the Analytical Interfering Factors That May Lead to Falsely Elevated hCG Results?

Some of the analytical interfering factors that may lead to falsely elevated hCG results include but are not limited to:

- heterophile antibodies, human anti-animal antibodies, rheumatoid factor, and autoantibodies[11]
- fibrin clots in serum as a result of incompletely clotted specimens
- interference from other endogenous components in the blood such as bilirubin, hemoglobin, and lipids[12]

## 7. What Are the Endogenous Interfering Antibodies (Heterophile and Human Anti-Animal Antibodies)?

Heterophile antibodies are produced against poorly defined antigens, frequently foreign proteins. The general term "heterophile antibodies" is sometimes used in the literature interchangeably to refer to heterophile antibodies, human anti-animal antibodies, rheumatoid factor, and autoantibodies.

Human anti-animal antibodies are circulating human antibodies reactive to animal proteins.[11] Circulating antibodies with specificities for a wide range of animal immunoglobulins have been reported such as mouse, rat, rabbit, and others.[11] The most common human anti-animal antibody interferent is human anti-mouse antibodies (HAMA), which causes both positive and negative interferences in mouse monoclonal antibody-based assays.[11]

Circulating heterophile antibodies and anti-animal antibodies have the potential to interfere with two-site (sandwich) or competitive immunoassays, such as hCG assays, by cross-linking the capture and label antibodies in the absence of specific analyte.[11,13] The estimated prevalence of interfering antibodies in the general population is up to 40% of normal serum samples.[13,14,15]

Most modern immunoassays contain nonspecific blocker immunoglobulins (which originate from the same species as the analyte-specific antibodies) in order to limit the effect of the interfering antibodies.[16] However, in some instances the blocking proteins can not sufficiently neutralize the interfering antibodies. Thus, analytical errors may occur.

An individual may acquire these antibodies from a variety of sources including the use of mouse monoclonal antibodies in diagnostic imaging and cancer therapy; exposure to microbial antigens; exposure of veterinarians, farm workers, and food preparers to foreign proteins; exposure of workers to animals in research laboratories and veterinary facilities; the presence of domestic animals in the home; autoimmune diseases which can give rise to autoantibodies such as rheumatoid factor; blood transfusion; vaccination; and maternal transfer across placenta to the fetus.[11,13,17]

hCG immunoassays may suffer from the interference of heterophile antibodies/human anti-animal antibodies/autoantibodies which in turn may lead to false-positive results.[3,18]

## 8. How Can Laboratorians Identify Potentially False-Positive Results in a Specimen Tested for hCG?

Laboratorians should suspect the occurrence of false-positive results if at least one of the following events occurs:[1,10,18]

- The blood test result is not reproducible on the same or different assay system.
- The blood test result is not linear after serial dilutions and rerun of the sample.
- The blood test produces a positive hCG result, but a parallel urine test produces a negative result. It is important to note that this criterion can be applied only to blood samples with hCG = 50 IU/L. The reason is that the sensitivity of urine tests is approximately 25 IU/L; therefore, if blood hCG values are less than 50 IU/L, urine levels may be too low to be detected.
- Test result turns negative following treatment of the sample with a heterophile antibody blocking agent.
- The patient's clinical presentation does not match the hCG positive result.

## 9. Can Results of Different hCG Assay Systems Be Compared?

For assay results to be easily comparable, they must measure the same molecular form of the analyte and be calibrated using the same reference material. There are standards issued by the World Health Organization (WHO) to calibrate immunoassays for hCG and its subunits[3]. All of the more recent hCG assays cleared by FDA are calibrated using one of the WHO standards.

In addition to regular hCG, there are at least five major variants of the hCG molecule present in serum and urine samples, plus a ß-core fragment which is only present in urine.[1,2] Although all of these forms are detectable in serum and/or urine samples during pregnancy, variable detection or lack of detection of cleaved molecules, free subunits, and fragments is a major cause of inter-assay variation in hCG results.[2,3]

Different hCG assays use antibodies to different sites on the hCG molecule.[18] Therefore, different tests may measure very different combinations of hCG-related molecules. This may not be important for the detection and monitoring of pregnancy since most hCG assays are designed to detect primarily placental hCG, which is the predominant hCG molecule form during pregnancy.[18] However, assay differences in terms of antibody binding sites may create variability in test results when the tests are used for detection and monitoring of other conditions such as hydatidiform moles or gestational trophoblastic diseases where other forms of hCG molecule are predominantly present.[18]

In addition, the size of hCG molecules circulating in the blood of individuals may vary as a result of differences in the protein and carbohydrate structure.[10] Also, some individuals

may produce aberrant forms of hCG that cross-react with the hCG assays.[10] Others may partly break down circulating hCG into non-biologically-active forms that react differently with the various assay systems.[10] In these conditions, other substances than native, biologically active hCG may be recognized by the assay system, and this can sometimes account for differences in measurements reported by different assays.[10]

## 10. If an hCG Test Result Does Not Match the Patient's Clinical Presentation, What Should Laboratorians Consider Doing?

If an hCG test result does not match the patient's clinical presentation (which will depend on the assay's intended use), it is possible that the test result is falsely elevated due to analytical interfering factors and/or device malfunction. The laboratory should investigate the presence of any interfering factors, the possibility of device malfunction, and verify the test result using the following guidelines.

The American College of Obstetricians and Gynecologists (ACOG), the Committee on Gynecologic Practice, published the following opinion in 2002[10] in order for laboratorians to rule out the presence of heterophile antibodies and other interfering substances in hCG immunoassays.

a. If the serum value of hCG is =50 IU/L, a quantitative or qualitative hCG urine test can be performed to rule out the presence of heterophile antibodies. Because heterophile antibodies are not present in urine, if the urine test is negative and the serum test is persistently positive, it confirms the serum immunoassay interference.

b. Wide variations between repeat runs of the same assay could result from the presence of interfering factors. The assay can be rerun with serial dilutions of the serum. Heterophile antibodies are directed to reagents in the immunoassay and not to hCG molecules so their interaction with the hCG curve will not be linear. Lack of linearity confirms assay interference.

c. Assay malfunction due to various inherent assay factors can result in false-positive results. Repeating the test using a different assay system will confirm a false-positive result if the repeat result is negative.

d. The serum can be pre-absorbed to remove heterophile antibodies before performing the hCG assay. The interference is confirmed if the result becomes negative after pre-absorbing the serum.

e. Because of the presence of hCG molecules with different sizes, aberrant forms of hCG, and non-biologically active forms of hCG circulating in blood of different individuals, the amount of true hCG may be measured differently or incorrectly by different immunoassays. Repeating the hCG test in a different assay system can detect this problem.

f. Patients with evidence of hCG assay interference should be notified of the risk for recurrent false-positive results. These patients should be instructed to inform their future health care providers of this issue, and the information should be recorded in their medical records.

In addition to the ACOG Committee's opinion, the following methods[11] may also be applied to reduce or remove the effect of interfering antibodies in hCG assays after ruling out the possibility of technical errors and analyzer malfunction:

- Use commercially available heterophile blocking reagents.
- Remove endogenous immunoglobulins by adding endogenous immunoglobulin-free serum samples to the specimen. For example, use normal mouse (animal) sera as blocking reagent.

**11. If an hCG Test Result Does Not Match the Patient's Clinical Presentation, What Should Physicians Consider Doing?**

In cases when an hCG test result does not match the patient's clinical presentation, it is important that the physician gather all the available information and reassess the patient. The physician may:

- consider the possibility that some other clinical condition may be causing an elevated hCG level
- communicate with the laboratory staff about the test result and ask the laboratory to rule out technical errors, analytical interfering factors, and device malfunction.
- consider repeating the blood draw and retesting
- consider testing with urine
- review the clinical presentation and consider additional diagnostic testing and bear in mind that the hCG test result is only one piece of the diagnostic puzzle.

**12. Is It Important That Laboratorians Follow Manufacturer's Recommended hCG Assay Instructions?**

Yes. It is critical that laboratorians follow manufacturer's assay instructions for use found in the device package insert. It is important that patient specimens be collected and processed according to the manufacturers' recommendations because improper collection, handling, and preparation of specimens can impact the accuracy of results. The following general recommendations are especially useful in order to avoid false-positive results due to interfering factors:

- Store unused collection tubes and blood specimens according to the manufacturers' recommendations.
- Follow manufacturers' instructions for using collection tubes with anticoagulants. Some may contain insufficient anticoagulant and lead to elevated or decreased results.

- Mix the content of tubes properly at the time of blood collection to prevent incomplete clot formation (serum) and platelet clumping or clotting (plasma).
- Process specimens according to the tube manufacturer's recommendations. Different types of tubes may have different requirements.
- Use a refrigerated, horizontal centrifuge head for best results. Use the centrifuge settings recommended by the tube manufacturer.
- Inspect samples for clots, fibrin, particulate matter, and other debris prior to processing them on an analyzer. Cellular debris from grossly hemolyzed samples may elevate test results.
- Follow the manufacturers' recommended calibration and/or maintenance schedules since analyzer malfunction is one of the common assay interfering factors that leads to inaccurate results.

## 13. Reporting to FDA

If there are any questions or concerns regarding the performance of hCG test method, contact the assay manufacturer. You should report all occurrences of unusual test performance to the manufacturer, and you are encouraged to also report them to FDA. To obtain more information about medical device reporting you can refer to the FDA's web site at **http://www.fda.gov/cdrh/mdr/index.html**.

### A. All Reports Should Be Sent to:

Food and Drug Administration
Center for Devices and Radiological Health
Medical Device Reporting
P.O. Box 3002
Rockville, MD 20847-3002

### B. For any Questions or Concerns Regarding the Content of This Communication, You May Contact:

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